·Basic Research·

Retinal vessel diameter changes induced by transient high perfusion pressure

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Received: 2014-01-21 Accepted: 2014-04-09

Abstract

•AIM: To investigate the effects of transient high perfusion pressure on the retinal vessel diameter and retinal ganglion cells.

•METHODS: The animals were divided into four groups according to different infusion pressure and infusion time (60 mm Hg–3min, 60 mm Hg–5min, 100 mm Hg–3min, 100 mm Hg–5min). Each group consisted of six rabbits. The left eye was used as the experimental eye and the right as a control. Retinal vascular diameters were evaluated before, during infusion, immediately after infusion, 5min, 10min and 30min after infusion based on the fundus photographs. Blood pressure was monitored during infusion. The eyes were removed after 24h. Damage to retinal ganglion cell (RGC) was analyzed by histology.

•RESULTS: Retina became whiten and papilla optic was pale during perfusion. Measurements showed significant decrease in retinal artery and vein diameter during perfusion in all of the four groups at the proximal of the edge of the optic disc. The changes were significant in the 100 mm Hg–3min group and 100 mm Hg–5min group compared with 60 mm Hg–3min group ($P1$=0.025, $P2$=0.000). The diameters in all the groups recovered completely after 30min of reperfusion. The number of RGC)showed no significant changes at the IOP in 100 mm Hg with 5min compared with contralateral untreated eye (P>0.05).

•CONCLUSION: Transient fluctuations during infusion lead to temporal changes of retinal vessels, which could affect the retinal blood circulation. The RGCs were not affected by this transient fluctuation. Further studies are necessary to evaluate the effect of pressure during real–time phacoemusification on retinal blood circulation.

•KEYWORDS: ocular perfusion pressure; retinal ischemia; retinal ganglion cells; animals

DOI:10.3980/j.issn.2222–3959.2014.04.03

Zhao YY, Chang PJ, Yu F, Zhao YE. Retinal vessel diameter changes induced by transient high perfusion pressure. *Int J Ophthalmol* 2014;7(4):602–607

INTRODUCTION

Improvement in the techniques of phacoemulsification has become one of the most gratifying to patients. Because of the extraordinary high expectations from the patients, cataract surgeons do their best to lower the direct injures from phacoemulsification. The application of maximum vacuum to reduce the power of phacoemusification is the efficient method to rapidly complete the surgery with lowest power. Nevertheless most of surgeons ignore the high perfusion pressure induced by maximum vacuum. It has been discussed at length by Khng *et al* [1] that the intraocular pressure (IOP) fluctuated from 66.1 to 196.6 mm Hg during nuclear disassembly of the surgery on cadaver eyes. Similarly, studies done in the dynamic changes *in vivo* during three simulated steps of phacoemulsification. The author described that the static IOP increased to 96 mm Hg during cortical cleanup, while the dynamic IOP fluctuated to 74 mm Hg [2]. Other studies reported that ocular hemodynamics changed after uncomplicated cataract surgeries[3]. Additionally, case reports and several clinical researches indicated that cataract surgery may be associated with an increased incidence of nonarteritic anterior ischemic optic neuropathy (NAION) [4-7]. These authors hypothesize that the high IOP associated with cataract surgery may be a factor to result of NAION. However there is no detailed evidences about the potential risk of high perfusion pressures during phacoemulsification.

Clinically, determination of the changes in blood circulation after transient IOP is important for understanding the effects of IOP increase with phacoemusification surgery, especially for doctors who are not very practiced in phacoemusification. Thus, the purpose of this study was to investigate the effects of transient high perfusion pressure on retinal blood vessels and retinal ganglion cells.

MATERIALS AND METHODS

Animal Preparation Experiments were conducted in strict accordance with the ARVO statement for the use of animals in ophthalmic and vision research. Twenty-four male pigmented rabbits weighing 2.5-3.5 kg were purchased from

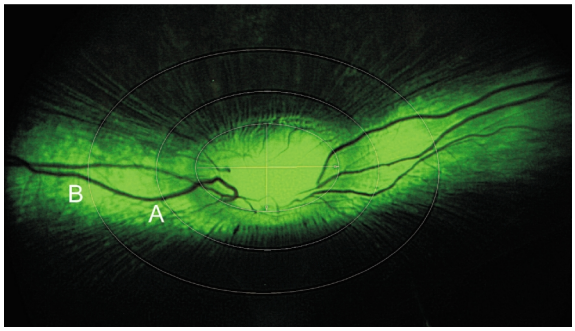

Figure 1 Fundus image from one of the examined rabbits Zone A is 0.5 disc diameters from the optic disc margin, and zone B is 1.0 to 1.5 disc diameters from the optic disc margin. Retinal vessel diameter measurements are performed around zone A and B.

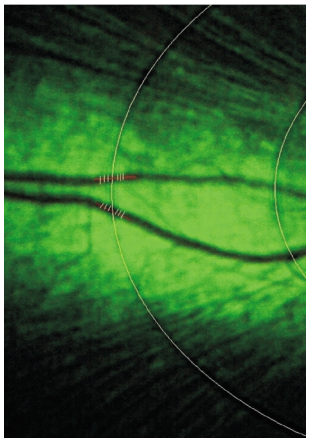

Figure 2 Digitized retinal photograph showed the retinal vessel diameter measurements. The average widths of 6 measures of a particular vessel segment are measured.

Wenzhou medical college animal laboratory Supplies (Wenzhou, China). Anesthesia was induced with an intramuscular injection of ketamine 36 mg/kg and xylazine 5 mg/kg. We topically anesthetized the cornea with proparacaine 0.5% and dilated the pupil with topical tropicamide 1%. The left eye of each rabbit was used as the experimental eye and the right as a control. This study was approved by Animal Ethics Committee of Wenzhou Medical University.

Models of Transient High Intraocular Pressure The transient high infusion model was made by a 16 gauge needle inserted into the anterior chamber at the limbus. Through a 3-way stopcock, the needle was connected to a bottle of balanced salt solution and to a second transducer connected to a digital monitor spontaneously. By this method a constant intraocular pressure could be maintained and monitored. The IOP was measured by tonopen tonometer (Mentor Tono-Pen XL, Medtronic Solan, USA). Based on it, the height of the bottle was set in advance. Thus we could precisely adjust the IOP to achieve what we want expeditiously. Animals were divided according to different infusion pressure and time (60 mm Hg-3min, 60 mm Hg-5min, 100 mm Hg-3min, 100 mm Hg-5min). Each group consisted of six eyes.

Retinal Vessel Caliber Measurement Color fundus photographs were taken after dilating the pupils with 1% tropicamide and 2.5% phenylephrine hydrochloride through a green filter using a fundus camera (NIKON 505, Nikon Corporation, Tokyo, Japan). To get the good-quality images, we used the balanced salt solution as the perfusion solution and kept the cornea moist with artificial tears during perfusion. Primary retinal images were obtained first. After that fundus photographs were captured during the infusion, immediately after perfusion, 5, 10 and 30min after infusion. Retinal vascular caliber was analyzed using customized imaging software system (Image-Pro Plus ver. 4.0; Media Cybernetics Inc., MD, USA). The measurement was located at a specified area [0.5-1 disc diameter (zone A)] which was distal from the optic disc and 1.0-1.5 (zone B) which was proximal the optic disc (Figure 1). The operator selected the edge of zone A and zone B for measurement. The average of 6 measurements was taken (Figure 2). Thirty pieces of these pictures (containing different period) were measured on two occasions 1wk apart by two raters trained in this method to ensure the reliability. Based on the result of reliability, all retinal measurements were performed by a single person in a masked fashion.

Histological Analysis of Retina The animals were killed after 1d of perfusion. After enucleated on both, the eyes were fixed in 2% paraform-aldehyde and 2.5% glutaraldehyde in 10 mmol/L phosphate-buffered saline over 24h. Then manual dehydrated in a series of graded alcohol solutions and embedded in paraffin. Oriented tissue sections (5 μm) vertically were cut parallel to the medullary rays. The sections involved the center of optic nerve head (ONH) were cut and stained with hematoxylin and eosin (H&E), then examined by light microscopy. Cells in the ganglion cell layer (GCL) of the retina were counted by one masked examiner to evaluate the damage to the retina. The examiner counted all of the ganglion cells at a distance between 1 and 1.5 mm away from the optical disk. Eight sections from each specimen were evaluated in the light microscopy at the magnification of 40 and the mean values were calculated for each group.

Statistical Analysis Data were analyzed using SPSS 12.0 for Windows. Analysis of variance between the eyes was made with a paired t-test. $P<0.05$ being considered statistically.

RESULTS

***In Vivo* Observation of the Retinal Vessel** Figure 3 provided individual example as the perfusion pressure of 100 mm Hg keeping 5min. Retinal whitening was observed around the optic disk and medullary wings and accompanied with collapsed retinal vessel during the perfusion, especially around the optic nerve head. Furthermore the cup of the optic nerve head become deeper and larger. Immediately pulling out the infusion needle which was similarly like the motions

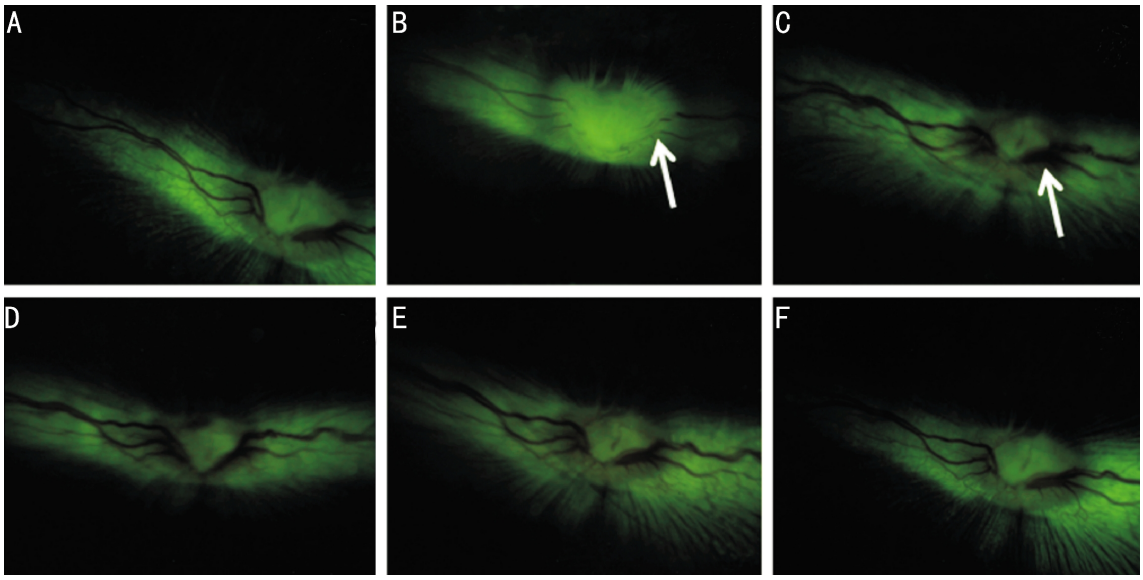

Figure 3 Retina fundus photographs were taken in different time points in the left eye of a rabbit Before (A) and during (B) and after perfusion in the 0, 5, 10 and 30min (C, D, E, F).

during the phacoemusification surgery, there was a mild reactive hyperemia and the mild reperfusion recovered after 30 min.

We evaluated the alterations of retinal vessel diameter as percentage changes of the initial value. The results showed a significant decrease in retinal artery during perfusion in all of the four groups. The changes were significant in the 100 mm Hg-3min group and 100 mm Hg-5min group compared with 60 mm Hg-3min group ($P1$=0.025, $P2$=0.000). The diameter of proximal retinal artery recovered completely after 30min of reperfusion statistically (Figure 4). Furthermore, the decrease of retinal artery diameter at the distal of the optic disc was up to 42.01% when the IOP is 100 mm Hg with 5min (Figure 5). Eventhough the changes after the perfusion instantly were significant in the four groups, the diameters recovered gradually after 10min (Figure 3A). Changes of retinal vein diameter varies greatly in the reperfusion time among individuals. Unlike the changes near the optical nerve head, diameters of retinal artery away from optical nerve head changes smoothly, especially in the reperfusion time. The date showed no statistically significance (Table 1).

The results of factorial analysis showed that there was interaction between perfusion pressure and perfusion time (F =11.929, P =0.001). Furthermore perfusion pressure impacted more than perfusion time ($P1$=0.007, $P2$=0.752).

Histological Analysis The number of retinal ganglion cell (RGC) showed no significant changes at the IOP in 100 mm Hg with 5min compared with contralateral untreated eye (P>0.05) (Figure 6).

DISCUSSION

The purpose of this study was to investigate the effects of transient high perfusion pressure on retinal blood vessels and retinal ganglion cells. Rabbits were chosen because the arterial supply of the ONH was relatively similar with that in humans [8]. According to the research by Kaja *et al*[9], oestrogen made the role of neuroprotective on retinal neurons, therefore male rabbits were selected in our research. We focused on short pressure transients reaching values (60 and 100 mm Hg) that can be experienced in phacoemulsification surgery [2]. Despite of different methods used to evaluate the blood circulation of retina, we choose vessel diameter as the indicator, which could reflect the change of blood flow and autoregulation conveniently.

Autoregulation During Infusion Based on the concept that the function referred to as autoregulation, blood flow in retina is stably maintained despite certain fluctuation in ocular perfusion pressure (OPP). However autoregulation insufficiency leaded inadequate response to changes in perfusion pressure which may induce ischemia. It has been shown previously in numerous animal and human experiments that ONH and retinal blood flow decrease after changes in OPP [10-12]. Geijer and Bill [13] reported that retinal ischemia appearance when IOP was elevated to a level that retinal perfusion pressure was less than approximately 20 mm Hg. The electroretinogram experiment revealed that pattern electroretinogram amplitude decreased significantly as the IOP elevated to 50 mm Hg[14]. Yang *et al*[15] found that autoregulatory capacity was greatly limited when IOP was over 40 mm Hg. Our findings agree with these observations: when the IOP was rapidly increased from a pre-elevated level, the diameter of retinal blood vessel decreased significantly during perfusion, especially in the artery at the proximal location (35.38%, 27.68%, 18.7%, 52.42% compared with before). Furthermore, artery at proximal changes more significant with the distal artery during acute IOP elevation in 100 mm Hg-5min group (P=0.009, Table 1). The result indicated that artery close to the ONH was

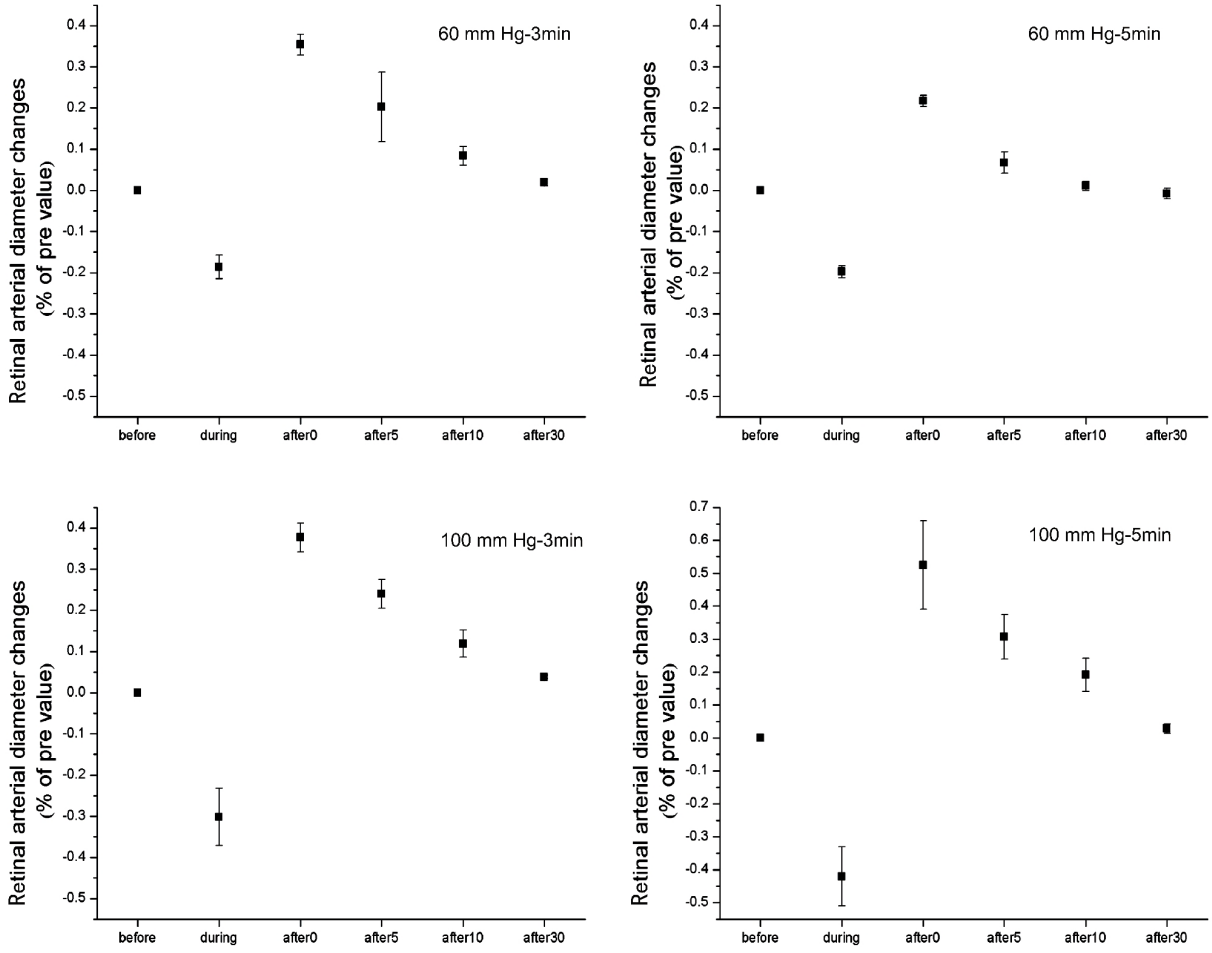

Figure 4 Scatterplots (mean±SE) demonstrating changes of retinal artery vessels at the proximate of the optic disc in different groups (60 mm Hg-3min, 60 mm Hg-5min, 100 mm Hg-3min, 100 mm Hg-5min).

Table 1 Changes of retinal blood vessels during perfusion

Groups	Artery (Proximal)				Vein (Proximal)			
	Before (pixel)	During (pixel)	Δ (%)	*P*1	Before (pixel)	During (pixel)	Δ (%)	*P*1
60 mm Hg-3min	20.07±6.10	16.07±3.84	18.66%±0.02	0.025	24.55±5.58	20.05±4.69	17.86%±0.01	0.007[a]
100 mm Hg-3min	14.23±4.02	9.73±1.84	30.19%±0.06	0.011	20.30±2.71	14.48±3.56	28.15%±0.01	0.002[a]
60 mm Hg-5min	14.59±2.73	11.80±2.92	19.80%±0.01	0.000[a]	24.63±2.54	20.12±2.14	18.24%±0.04	0.001[a]
100 mm Hg-5min	14.86±3.37	8.36±1.10	42.01%±0.03	0.007[a]	21.22±3.22	13.15±2.45	36.09%±0.09	0.013

Table 1 Changes of retinal blood vessels during perfusion (continued)

Groups	Artery (Distal)				Vein (Distal)				*P*2	*P*3
	Before(pixel)	During(pixel)	Δ (%)	*P*1	Before(pixel)	During(pixel)	Δ (%)	*P*1		
60 mm Hg-3min	20.27±5.62	17.16±3.69	14.22%±0.06	0.039	24.95±2.61	20.47±4.73	15.83%±0.09	0.018	0.080	0.995
100 mm Hg-3min	15.47±4.74	10.57±2.16	30.05%±0.07	0.014	21.77±2.57	14.61±1.95	32.70%±0.07	0.001	0.848	0.123
60 mm Hg-5min	12.99±1.64	9.14±1.67	19.45%±0.05	0.001[a]	18.03±2.76	13.06±2.99	26.99%±0.05	0.025	0.067	0.334
100 mm Hg-5min	16.92±3.16	13.28±3.14	21.73%±0.08	0.007[a]	22.12±2.06	17.63±1.47	22.88%±0.08	0.008	0.009[a]	0.033

*P*1: Statistical analysis with paired *t* test between before and during of the high infusion at artery and vein; *P*2: Statistical analysis with paired *t*-test between proximal and distal at artery; *P*3: Statistical analysis with paired *t*-test between proximal and distal at vein; [a]*P*<0.01
Δ=[(before-during)/before]%

vulnerable to severe vascular insults. Eventhough we can't find a significant constriction in retinal artery compared with the retinal vein in the same location in all of the groups, the changes in the vein at the proximal location during perfusion were smooth. We considered that it was because retinal vein had the weaker wall construction and the lower intravasal pressure of the venous system. Additionally, we observed significant decrease of blood vessels, without a complete obstruction in the major blood vessels following with collapses of capillary. It was because erythrocyte is mainly distributed at the center of blood vessl, while plasma located near the vessel walls during blood flow. Shonat *et al*[16]

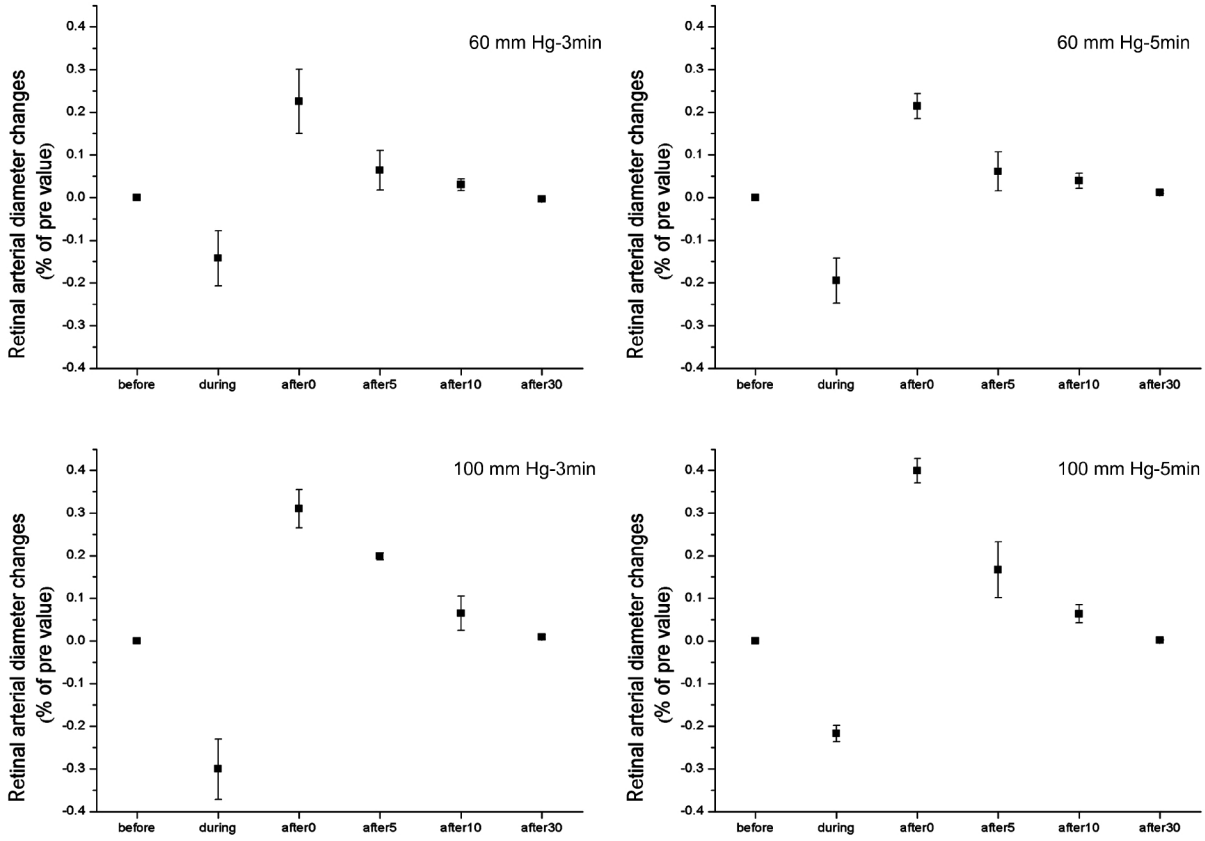

Figure 5 Scatter plots (mean±SE) demonstrating changes of retinal artery vessels at the distal of the optic disc in different groups (60 mm Hg−3min, 60 mm Hg−5min, 100 mm Hg−3min, 100 mm Hg−5min).

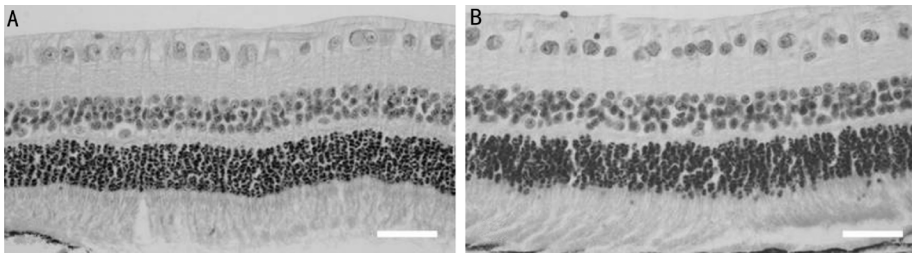

Figure 6 Representative photomicrographs showing histological appearance of the 100 mm Hg−5min group A: Experimental eye; B: Control eye. Scale bars: 100 μm.

reported that hypoxia only happened as the blood flow was stopped. So we speculated despite without obstruction of blood vessel, the functional blood flow may be stopped and capillary regions are more serious than major blood vessels regions.

The release of the high pressure occurred suddenly when the needle was pulled out. Figure 4 also illustrated the change after drawing out the needle from the anterior chamber. Mild dilation was seen in our study. The hyperemia showed no significant change compared with before in all groups. Furthermore this reactive hyperemia was recovered within 30min.

Our results reveal the procedure of autoregulation in different infusion pressure in normal rabbits. As autoregulatory capacity couldn't tolerate the infusion, it will certainly result in impairment of blood flow[17].

Morphology of Retinal Ganglion Cell It has been noted that ganglion cells are the most susceptible to ischemia of the retina [18,19]. Szabo *et al* [20] reported that ischemia for less than 60min followed by reperfusion causes no histologic changes in the retina. However Resta *et al* [18] findings revealed that rapid pressure transients induce RGC injury within hours. It has been proposed that ganglion cell loss after retinal ischemia is an ongoing process, for which severity and duration are determined by the ischemic interval[21]. Our study shows that infusion tolerance time of 5min with 100 mm Hg have no threat to the structure of ganglion cells. We proposed that transient acute IOP elevation didn't induce retinal ganglion cell damage.

Several limitations of this study should also be noted. The first limitation concerns the manual determination of diameter of the retinal blood vessels. The semi-automatic method for determining the diameter was not strictly correct. We adopted double-mask method to minimize measurement

error. Secondly we just evaluated the effect of retinal circulation in simulated conditions of phacoemulsification. The effects in the actual procedure of phacoemusification should be done in the future.

In conclusion, transient high infusion pressure leads to temporal changes of retinal vessels, which affect the retinal blood circulation. It depends on different pressure parameters (duration, peak value). As mentioned previously, the present data should be interpreted with caution when applying them to the situation in patient with vascular dysregulation or with low perfusion pressure.

ACKNOWLEDGMENTS

Foundation: Supported by Natural Science Foundation of Zhejiang Province (No.LY12H12004)

Conflicts of Interest: Zhao YY, None; **Chang PJ,** None; **Yu F,** None; **Zhao YE,** None.

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